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Full Length Research

Ameliorative Potential of Bambara Nuts (*Vigna subterranea* (L.) Verdc) Extract against Acute Ethanol-Induced Oxidative Stress in Wistar Rats

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The aim of this study was to determine the ameliorative potential of Bambara nuts extract against acute ethanol-induced oxidative stress in Wistar rats. Bambara nuts of the Songkhla 1 variety locally sourced in Obinze area of Owerri, Imo State, Nigeria were powdered using a coffee grinder and an extract obtained from a Soxhlet apparatus with methanol as solvent. Twenty-four adult male Wistar rats were acclimatized for seven days and were randomly divided into four groups of six rats each. Animals in groups A and B were administered distilled water while those in groups C and D were administered Bambara nut extract for twenty-one days at a dose of 100 mg/kg body weight 12 hourly via oral route. At the end of the treatment, they were fasted overnight and animals in groups B and D were exposed to a single dose of 70% ethanol at 12 ml/kg body weight to induce oxidative stress. After 12 hours of ethanol administration, the animals were sacrificed and blood samples were collected via cardiac puncture. Oxidative stress parameters were determined using standard methods. Ethanol-induced oxidative stress significantly increased the activities of lipid peroxidation (LPO), catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) but decreased glutathione (GSH) concentrations. Bambara nut was able to remedy these effects by regulating the oxidative stress biomarkers, thus possesses ameliorative potential against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress. This also implies that it could boost the immune system. Thus, regular consumption of this nut is recommended.

Keywords: Ameliorative potential, Bambara nut, Ethanol, Oxidative stress biomarkers.

INTRODUCTION

Bambara nut (*Vigna subterranea* (L.) Verdc), is an African native legume high in protein and minerals (Murevanhema and Jideani, 2013). Its flour is widely used as a food source mainly for infants and

Figure 1. Bambara Nut.

youngsters. The high nutritional composition promotes its utilization in many food applications. Traditional processing of the seed includes dehulling, roasting or fermentation, and milling to obtain flour. The use of milk and flour obtained from Bambara nut is widely studied (Ijarotimi and Olopade, 2009; Ijarotimi et al., 2009; Falade et al., 2015; James et al., 2017). Other studies also addressed the potential of Bambara nut as composite in home-made weaning foods for infants and young (Figure 1). Bambara nut has been ranked as the third most important grain legume, after groundnut *(Arachis hypogaea L.)* and cowpea *(Vigna unguiculata)* in semi-arid Africa (Megwas et al., 2021).

It might be surprising to say that most people in Nigeria may not be conversant with the name Bambara nut as the local name is commonly used but it forms most parts of some families' daily meal. Locally, it is called 'Okpa' in Igbo, 'Epa-Roro' in Yoruba, 'Kwaruru' or 'Gurjiya' in Hausa (Megwas et al., 2021).

Excessive acute or chronic alcohol consumption poses a serious health hazard and can result into several metabolic disorders in organisms (Lieber, 2000). Alcohol is a commonly used hepatotoxin in experimental hepatopathy. The pathogenesis of alcohol-induced liver disease is not clearly defined, there is evidence that ethanol-induced liver injury is due to oxidative stress that leads to fibrosis and impaired liver functions (Wu and Cederbaum, 2003; Ronis et al., 2004). Alcohol overuse is also characterized by central nervous system (CNS) intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes (Gohlke et al., 2008). Excessive alcohol consumption commonly causes hepatic, gastrointestinal, nervous and cardiovascular injuries leading to physiological dysfunctions (Lieber, 1998). Cellular disturbances resulting from excessive alcohol consumption causes an increase in oxidative stress biomarkers such as malondialdehyde (MDA); reductions in glutathione level and activities of antioxidant enzymes (Nadro et al., 2007; Das and Vasudevan, 2007). Free radicals and reactive oxygen species (ROS) have been implicated in the oxidative damage of biomolecules and various organs of the body. Studies have shown the crucial role free radicals play in the pathogenesis of many human diseases namely, cardiovascular and pulmonary diseases, some types of cancer, immune/autoimmune diseases, inflammation, diabetes, cataracts and brain dysfunction such as Parkinson and Alzheimer (Rahman et al., 2012). Oxidative stress arises when there is an imbalance between free radical production (especially reactive oxygen species; ROS) and endogenous antioxidant defense system. This shift in balance is associated with oxidative damage to a wide range of biomolecules including lipids, proteins, and nucleic acids, which may eventually impair normal functions of various tissues and organs (Lobo et al., 2010). There is an increasing global interest concerning the use of medicinal plants in the prevention and treatment of different pathologies (Gorinstein et al., 2003; Ramana et al., 2014). The beneficial effects of plants are attributed to the presence of secondary metabolites such as polyphenols, tannins, terpenoids, alkaloids, flavonoids (Chikezie et al., 2015). Considering the central role played by free radicals in the initiation and progression of many diseases, the use of natural products with antioxidant constituents has been proposed as an effective therapeutic and/or preventive strategy against diseases and the search for potent and cost-effective antioxidants of plant origin has since increased (Morales-González, 2013).

Many plants have been shown to possess antioxidant potentials (Airaodion et al., 2019 a, b). This has thus raised interest in the investigation of commonly consumed plants for their phytochemicals with nutritional and chemotherapeutic potentials. Therefore, the need to argument synthetic chemotherapeutic compounds with natural products is the drive for the exploitation of natural products from plants; as they may have little or no side effects yet meeting the nutritional, chemotherapeutic

and economic needs (Airaodion et al., 2019 c, d). Moreover, despite the efforts of pharmaceutical companies in the production of synthetic antibiotics, there yet exists a marked increase in pathogen population exacerbated by multi drug resistant microorganisms. Consequently, there is increased research into phytochemicals for the effective therapeutics combat of this menace. The therapeutic effects of plant-based drugs have been documented to be due to the phytochemicals that constitute the plants (Airaodion et al., 2019 e, f). Recently, Megwas et al., (2021) reported that Bambara seeds possess hypoglycemic and hypolipidemic potentials. This present study is therefore aimed at examining the ameliorative potential of Bambara nuts against acute ethanol-induced oxidative stress in Wistar rats.

MATERIALS AND METHODS

Seed collection and seed extract

Bambara seeds of the Songkhla 1 variety (red seed coat) were locally sourced in Obinze area of Owerri, Imo State, Nigeria and were collected and properly identified by a botanist. Immature and damaged seeds were removed. Seeds were peeled, powdered using a coffee grinder and stored in screw-cap bottle at -20°C. Seed extract was obtained using soxhlet apparatus and methanol as the solvent (Airaodion et al. 2019g,h). About 25 g of seed powder and 250 mL of methanol were used for each 18 h distillation. Methanol was evaporated in a rotary evaporate at 35 ^{o}C to reach an extract-weight of 2.17 g, (about 8.68% of each sample distilled), extract was preserved in the refrigerator.

Treatments and Experimental Design

Twenty-four (24) adult male Wistar rats with body weight between 140 and 160 g were used for the experiment. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health (NAS, 2011). They were randomly divided into four (4) groups of six (6) rats each. Animals in groups A and B were administered distilled water while those in groups C and D were administered Bambara nut extract for twenty-one days at a dose of 100 mg/kg body weight 12 hourly via oral route of administration. Animals in group A served as the control group. At the end of the treatment, they were fasted overnight and animals in groups B and D were exposed to a single dose of 70% ethanol at 12 ml/kg body weight to induce oxidative stress. The dosage of ethanol used in this study has been documented to induce tissue toxicity and oxidative damage in rats (Airaodion et al., 2020a). After 12 hours of ethanol administration, the animals were anaesthetized using diethyl ether and were sacrificed and blood samples were collected *via* cardiac puncture.

Determination of Oxidative Stress Biomarkers

Blood concentrations of Lipid Peroxidation (LPO), Reduced Glutathione (GSH), activities of Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione peroxidase (GPx) were determined following the methods of Airaodion et al. (2019i).

Statistical Analysis

Results are expressed as mean ± standard error of the mean (S.E.M). The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant.

RESULTS

LPO concentration in rats given distilled water and ethanol (DWE) was about 1.8 times higher ($p<0.05$) than rats given Bambara seed extract and ethanol (BSEE), which also showed a similar LPO concentration than rats with no ethanol dosage (Figure 2). Administration of ethanol only decreased the concentration of GSH while Bambara nut only increased its concentration when compared with those in the control animals (Figure 3). Prophylactic treatment of animals in group D with Bambara nut

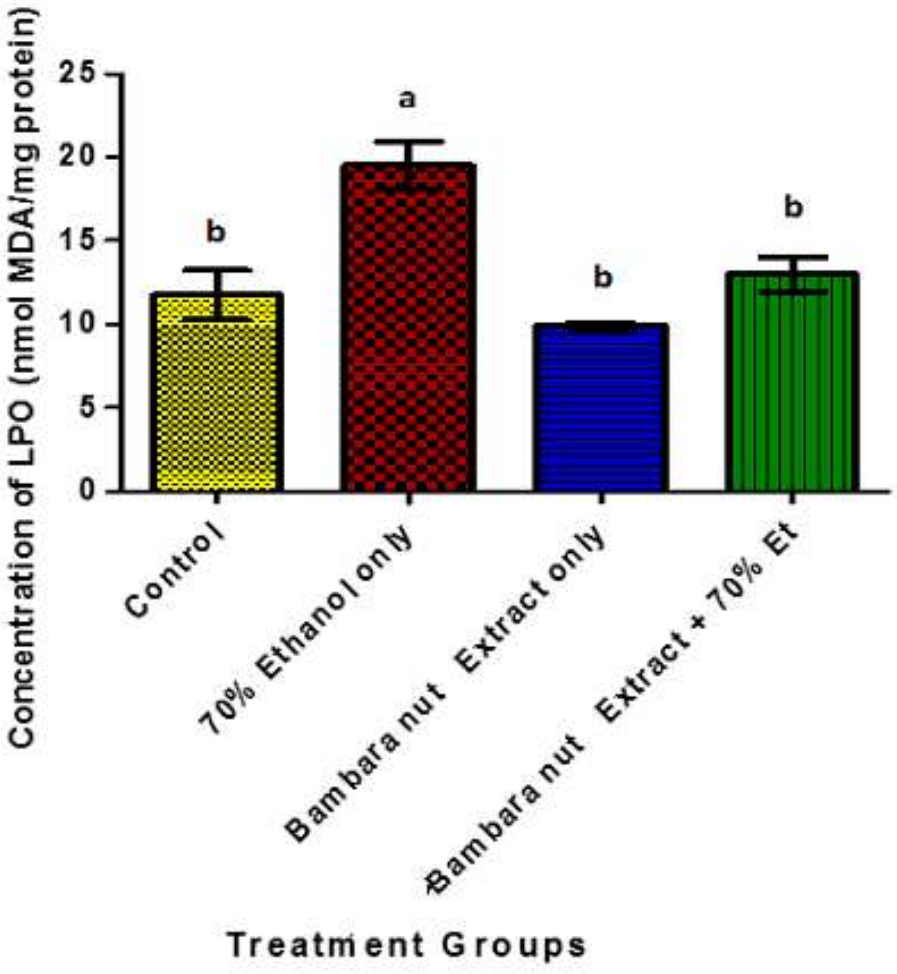

Figure 2. Effect of Bambara nut on Lipid Peroxidation (LPO) of Ethanol-induced Animals after 21 Days of Administration.

extract before ethanol induction attenuated the reduction in the concentration of GSH caused by ethanol. Treatment of animals with ethanol only increased the activity of CAT, SOD and GPx while Bambara nut only decreased their activities when compared with those in the control animals (Figures 4 - 6 respectively). Prophylactic treatment of animals in group D with Bambara nut extract before ethanol induction ameliorated the elevation in the activities of CAT, SOD and GPx caused by ethanol exposure.

DISCUSSION

Alcohol metabolism results in oxidative and nitrosative stress via elevation of NADH/NAD^+ redox ratios, induction of nitric oxide synthase (NOS) and NADPH/xanthine oxidase (Sathiavelu et al., 2009; Manzo-Avalos and Saavedra-Molina, 2010). Lipid peroxidation, a primary mechanism of cell membrane destruction and cell damage is a common feature of both acute and chronic alcohol consumption (Livingstone and Davis, 2007; Ramezani et al., 2012). The presence of a high concentration of oxidizable fatty acids and iron significantly contributes to ROS production. A rise in lipid peroxidation level is only identified if there is oxidative damage due to the increase in free radical generation. Generally, under normal conditions, the animals tend to maintain a balance between generation and neutralization of ROS in the tissues (Airaodion et al., 2020b). But, when the organisms are subjected to xenobiotic stress, the rate of production of ROS including O_2^-, H_2O_2, OH^-, ROO^{--},

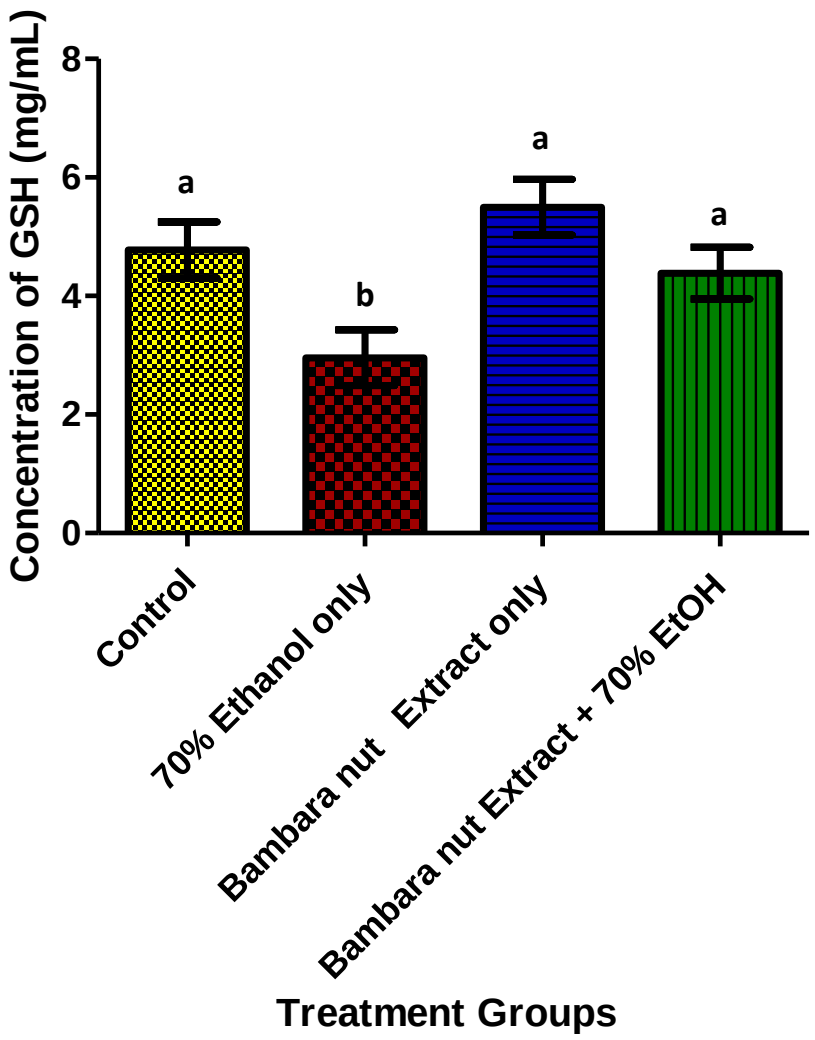

Figure 3. Effect of Bambara nut on the Concentration of Reduced Glutathione (GSH) of Ethanol-induced Animals after 21 Days of Administration.

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

exceeds their scavenging capacities (Airaodion et al., 2020c). All the organisms have their own cellular antioxidant defense system composed of both enzymatic and non-enzymatic components. Enzymatic antioxidant pathway consists of SOD, CAT and GPx. Superoxide anion O_2^- is dismutated by SOD to H_2O_2, which is reduced to water and molecular oxygen by CAT or is neutralized by GPX, which catalyzes the reduction of H_2O_2 to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. Glutathione reductase (GR) regenerates GSH from oxidized glutathione (GSSG), which is a scavenger of ROS as well as a substrate for other enzymes. Glutathione S-transferase (GST) conjugates xenobiotics with GSH for exclusion.

Rats dosed with ethanol without previous consumption of Bambara nut showed the highest malondialdehyde (MDA) levels and LPO activity which indicate an enhanced peroxidation and breakdown of the antioxidant defense mechanisms. Decomposition products of lipid hydroperoxide such as malanoldehyde and 4-hydroxynonenal, can cause chaotic cross-linkage with proteins and

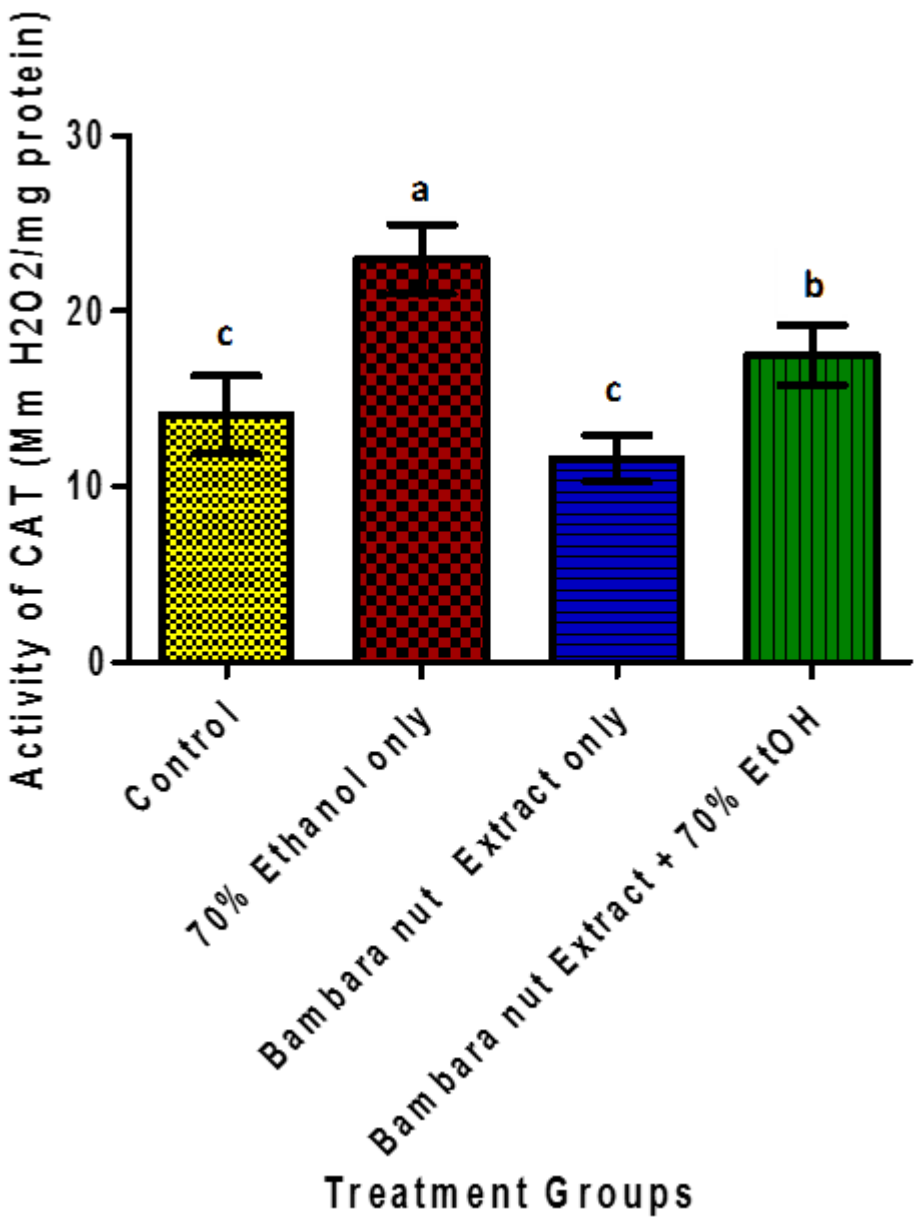

Figure 4. Effect of Bambara nut on the Activity of Catalase (CAT) of Ethanol-induced Animals after 21 Days of Administration.

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

nucleic acids, which plays an important role in the process of carcinogenesis (Airaodion et al., 2020a). Furthermore, extensive damage to tissues in a free radical mediated LPO results in membrane damage and subsequently decreases the membrane fluid content (Airaodion et al., 2019i). Controlled intake of Bambara seed before ethanol oral administration prevented the increase in MDA levels and LPO activity, suggesting its protective effects against ethanol-induced oxidative damage. This is consistent with the study of Airaodion et al. (2019i) who reported the hepatoprotective effect of *Parkia biglobosa* on acute ethanol-induced oxidative stress in Wistar rats. Animals treated with Bambara nut

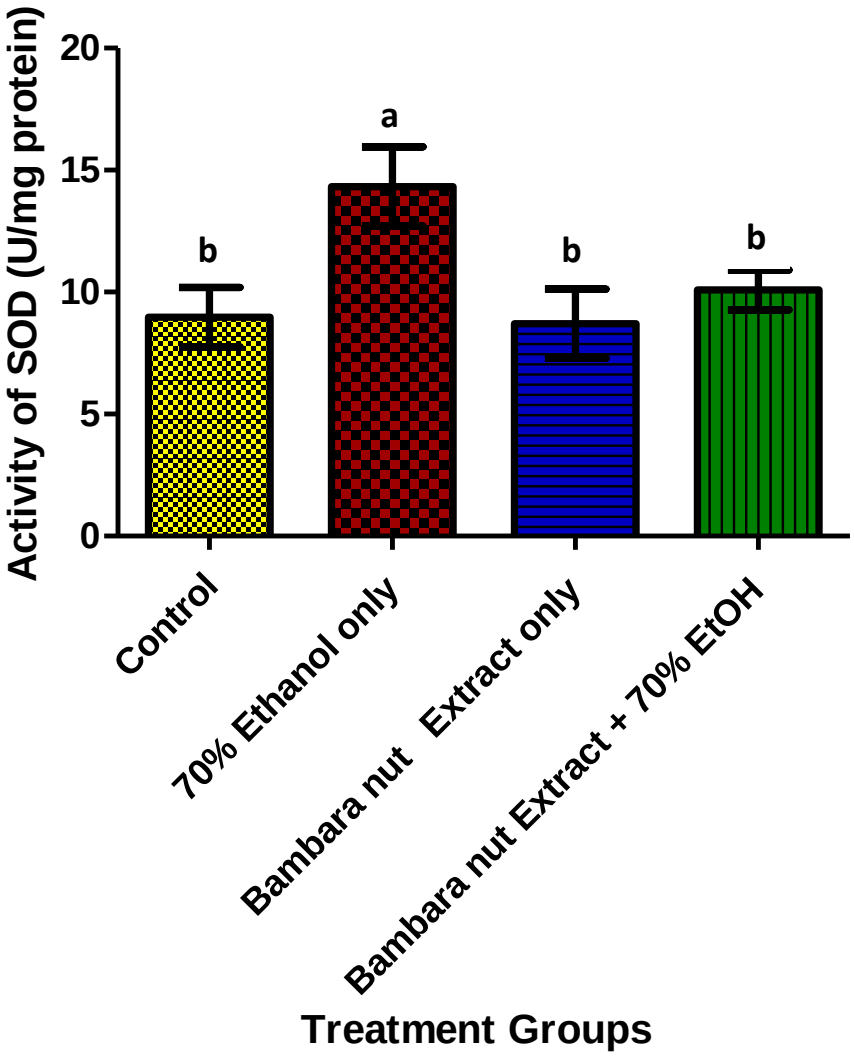

Figure 5. Effect of Bambara nut on the Activity of Superoxide Dismutase (SOD) of Ethanol-induced Animals after 21 Days of Administration.

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

without ethanol induction were observed to have reduced lipid peroxidation when compared with those administered ethanol as well as those in the control group (Figure 2). This is suggestive that the extract possesses antioxidant potential which helps in the reduction of lipid peroxidation.

Glutathione (GSH) is a tripeptide (L-α-glutamylcysteinol glycine) which is highly abundant in all cell compartments and it is the major soluble antioxidant. Glutathione directly quenches ROS such as lipid peroxides, and plays a major role in xenobiotic metabolism (Livingstone and Davis, 2007). Glutathione detoxifies hydrogen peroxide and lipid peroxide by donating electron to hydrogen peroxide to reduce it to water and oxygen protecting macromolecules such as lipids from oxidation. In this study, the decrease in the reduced glutathione level in animals treated with ethanol only is connected with ethanol-induced oxidative stress and direct conjugation of GSH with acetaldehyde and other reactive intermediates of alcohol oxidation. This result agrees with the findings of Airaodion et

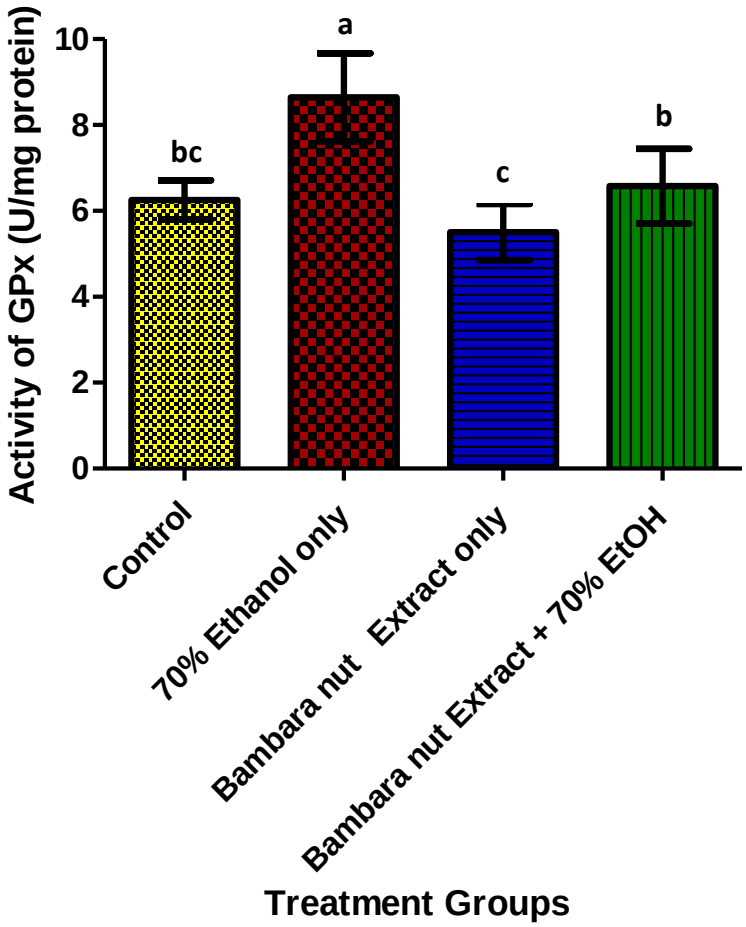

Figure 6. Effect of Bambara nut on the Activity of Glutathione Peroxidase (GPx) of Ethanol-induced Animals after 21 Days of Administration.

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

al. (2020a) who reported that acute ethanol treatment caused reduction in the glutathione levels in different tissues. The significant increase ($P < 0.05$) in the glutathione levels of Bambara nut-treated rats prior to ethanol-administration may be due to the direct ROS—scavenging effect of Bambara nut or an increase in GSH synthesis. This is consistent with the report of Airaodion et al. (2019j) who reported the ameliorative efficacy of methanolic extract of *Corchorus olitorius* leaves against acute ethanol-induced oxidative stress in Wistar rats.

Catalase (CAT) contributes to ethanol oxidation, by oxidizing a small amount of ethanol in the presence of a hydrogen peroxide (H_2O_2) generating system to form acetaldehyde (Agarwal, 2001). In this study, a significant increase was observed in the activity of catalase in control animals and those treated with Bambara nut extract without ethanol induction when compared with ethanol-induced animals with Bambara nut extract pretreatment. This contradicts the findings of Airaodion et al. (2019i) who reported a nonsignificant difference when animals were treated with *Parkia biglobosa*. The activity of catalase in animals pretreated with Bambara nut prior to ethanol induction was significantly reduced when compared with those without Bambara nut pretreatment. This might be an indication that ethanol-induced oxidative stress generated elevated ROS which CAT tend to

combat, thereby increasing its activity. Bambara nut was able to reduce the ROS generation with subsequent decrease in CAT activity due to its high phytochemical content (Nwadi et al., 2019) as well as its antioxidant potential reported by Chinnapun (2018). Increased CAT activity in this study following acute ethanol exposure suggests elevated ethanol oxidation and formation of oxidising product-acetaldehyde.

Superoxide dismutase (SOD) plays an important role in reducing the effect of free radicals' attack, and SOD is the only enzymatic system quenching O_2- to oxygen and H_2O_2 and plays a significant role against oxidative stress (Oh et al., 1998). These radicals have been reported to be deleterious to polyunsaturated fatty acids and proteins (Murray et al., 2003). In this study, no significant difference was observed in the activity of SOD in control animals and those treated with Bambara nut extract only when compared with ethanol-induced animals with Bambara nut extract pretreatment. The activity of SOD in animals pretreated with Bambara nut prior to ethanol induction was significantly reduced when compared with those without Bambara nut pretreatment. This might be that ethanol-induced oxidative stress generated elevated ROS which SOD tend to combat thereby increasing its activity. *Bambara nut* was able to reduce the ROS generation with subsequent decrease in SOD activity due to its high phytochemical content (Nwadi et al., 2019) as well as its antioxidant potential reported by Chinnapun (2018).

The increased activity of SOD observed in ethanol-induced animals contradicts the study of Halliwell and Gutterberidge (2006) who reported that SOD activity was considerably reduced during ethanol intoxication. Glutathione peroxidase (GPx) is another enzymatic antioxidant that acts as a defense against oxidative stress (Airaodion et al., 2020 b, c). In this study, no significant difference was observed in the activity of GPx in control animals and those treated with Bambara nut extract only when compared with ethanol-induced animals with Bambara nut extract pretreatment. The activity of GPx in animals pretreated with Bambara nut prior to ethanol induction was significantly reduced when compared with those without Bambara nut pretreatment. This might be suggestive that ethanol-induced oxidative stress generated elevated ROS in animals which GPx tend to combat thereby increasing its activity. Bambara nut was able to reduce the ROS generation with subsequent decrease in GPx activity due to its high phytochemical content (Nwadi et al., 2019) as well as its antioxidant potential reported by Chinnapun (2018). The increased activity of GPx observed in ethanol induced animals contradicts the studies of Airaodion et al. (2019i) who observed no significant difference in the activity of GPx in the study of hepatoprotective effect of *Parkia biglobosa* against acute ethanol-induced oxidative stress in Wistar rats and that of Yang et al. (2005) who also observed a non-significant difference in GPx activities in rats hepatocyte exposed to varying concentrations of ethanol at an incubation time of 12 hours. The toxicity of ethanol is related to the product of its metabolic oxidation. Acetaldehyde and acetate, produced from the oxidative metabolism of alcohol can form adducts with cellular macromolecules, causing oxidative damage and affecting metabolic processes (Shirpoor et al., 2008). Catalase and glutathione peroxidase further detoxify H_2O_2 into H_2O and O_2 (Airaodion et al., 2020b). Thus, SOD, catalase and GPx function mutually as enzymatic antioxidative defense mechanism to counter the deleterious effect of ROS. The effect of Bambara nut against alcohol-induced oxidative stress observed in this study is consistent with the findings of Chinnapun (2018) who reported that Bambara nut has the capacity to protect plasmid DNA against 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative damage.

CONCLUSION

Controlled intake of Bambara seed extract develops in male adult Wistar rats' protection against acute ethanol-induced oxidative stress providing information for further studies on the beneficial effects of Bambara seed extract on the preservation and enhancement of human health.

ETHICAL CONSIDERATION

Animal ethic Committee approval has been collected and preserved by the authors**.**

CONFLICT OF INTERESTS

Authors wish to declare that no conflict of interests exist in this study and publication.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Airaodion, A. I. designed the study and wrote the manuscript. Megwas, A. U. carried out the analyses of the study. Njoku, O. C. managed the literature searches while Oladosu, N. O. managed the statistical analysis. All authors read and approved the final manuscript.

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